BLEEP—Potential of Mean Force Describing Protein–Ligand Interactions: II. Calculation of Binding Energies and Comparison with Experimental Data

JOHN B. O. MITCHELL,[1] ROMAN A. LASKOWSKI,[2] ALEXANDER ALEX,[3] MARK J. FORSTER,[4] JANET M. THORNTON[1, 2]

[1] *Department of Biochemistry and Molecular Biology, University College London, London, UK*
[2] *Department of Crystallography, Birkbeck College, University of London, London, UK*
[3] *Computational Chemistry, Pfizer Central Research, Sandwich, Kent, UK*
[4] *Informatics Laboratory, National Institute for Biological Standards and Control, South Mimms, Herts, UK*

Received 18 January 1999; accepted 29 March 1999

ABSTRACT: We have developed BLEEP (biomolecular ligand energy evaluation protocol), an atomic level potential of mean force (PMF) describing protein–ligand interactions. Here, we present four tests designed to assess different attributes of BLEEP. Calculating the energy of a small hydrogen-bonded complex allows us to compare BLEEP's description of this system with a quantum-chemical description. The results suggest that BLEEP gives an adequate description of hydrogen bonding. A study of the relative energies of various heparin binding geometries for human basic fibroblast growth factor (bFGF) demonstrates that BLEEP performs excellently in identifying low-energy binding modes from decoy conformations for a given protein–ligand complex. We also calculate binding energies for a set of 90 protein–ligand complexes, obtaining a correlation coefficient of 0.74 when compared with experiment. This shows that BLEEP can perform well in the difficult area of ranking the interaction energies of diverse complexes. We also study a set of nine serine proteinase–inhibitor complexes; BLEEP's good performance here illustrates its ability to determine the

Present address: J. B. O. Mitchell, Department of Chemistry, University College London, 20 Gordon Street, London WC1H 0AJ, UK; e-mail: mitchell@biochemistry.ucl.ac.uk

Correspondence to: J. M. Thornton; e-mail: thornton@biochemistry.ucl.ac.uk

Contract/grant sponsor: Pfizer, Ltd.

CCC 0192-8651 / 99 / 111177-09

relative energies of a series of similar complexes. We find that a protocol for incorporating solvation does not improve correlation with experiment. © 1999 John Wiley & Sons, Inc. J Comput Chem 20: 1177–1185, 1999

Keywords: potential of mean force; knowledge-based potential; protein–heparin binding; protein–ligand interactions; computer-aided drug design

Introduction

As detailed in the accompanying study,[1] we have generated BLEEP (biomolecular ligand energy evaluation protocol), a potential of mean force (PMF) describing protein–ligand complexes, from data in the Brookhaven Protein Data Bank (PDB).[2] Two versions of BLEEP exist. BLEEP-1 includes only protein–ligand and water–ligand interactions from the crystal structures, whereas BLEEP-2 includes data from a computationally added shell of water molecules around each structure and incorporates protein–water and water–water interactions. Our preliminary tests have suggested that BLEEP-2 gives the more reliable and useful results. We therefore concentrate mainly on BLEEP-2 in this work.

Protocols for Use of BLEEP-2

Using BLEEP-2, one can first calculate a simple ''pseudo-gas-phase'' interaction energy for a protein–ligand complex. The interaction energies thus produced describe two molecules interacting only with each other. Although we call these ''pseudo-gas-phase'' energies, it is important to remember that BLEEP-2 was derived from data incorporating solvent molecules.

Second, one can, optionally, calculate solvent-inclusive interaction energies. Our algorithm for calculating the binding energy in the presence of water is based on the following equation. In eq. (1), note that the superscript ''**c**'' refers to the complex (final state), superscript ''**i**'' to the initial state (separated and solvated protein and ligand), subscript ''**p**'' to protein, subscript ''**l**'' to ligand, and subscript ''**s**'' to solvent. $\mathbf{\Delta W}$ refers to the change in interaction energy calculated using this potential, in a manner similar to that of Mitchell and Price.[3] The exact thermodynamic meaning of mean force potentials is often thought to be unclear, but we note Sippl's identification[4,5] of this formalism with the Helmholtz free energy, ΔA:

$$\Delta \mathbf{W} = \mathbf{W}_{\mathbf{pl}}^{\mathbf{c}} + \mathbf{W}_{\mathbf{ps}}^{\mathbf{c}} + \mathbf{W}_{\mathbf{ls}}^{\mathbf{c}} + \mathbf{W}_{\mathbf{ss}}^{\mathbf{c}} - \mathbf{W}_{\mathbf{pl}}^{\mathbf{i}} - \mathbf{W}_{\mathbf{ps}}^{\mathbf{i}} - \mathbf{W}_{\mathbf{ls}}^{\mathbf{i}} - \mathbf{W}_{\mathbf{ss}}^{\mathbf{i}} \quad (1)$$

Because the protein and ligand do not interact in the initial state, $\mathbf{W}_{\mathbf{pl}}^{\mathbf{i}} = 0$:

$$\therefore \Delta \mathbf{W} = \mathbf{W}_{\mathbf{pl}}^{\mathbf{c}} + \left(\mathbf{W}_{\mathbf{ps}}^{\mathbf{c}} - \mathbf{W}_{\mathbf{ps}}^{\mathbf{i}}\right) + (\mathbf{W}_{\mathbf{ls}}^{\mathbf{c}} - \mathbf{W}_{\mathbf{ls}}^{\mathbf{i}}) + (\mathbf{W}_{\mathbf{ss}}^{\mathbf{c}} - \mathbf{W}_{\mathbf{ss}}^{\mathbf{i}}) \quad (2)$$

The first term is simply the ''pseudo-gas-phase'' protein–ligand interaction energy, the second is the change in protein–solvent interaction energy on complex formation, the third is the change in ligand–solvent energy, and the final term is the change in solvent–solvent energy. One can calculate the first three terms directly from the initial and final pseudo-gas-phase energies of protein–ligand, protein–water, and ligand–water interactions. The protein–water interaction energy in a given state is the sum of the pairwise interaction energy of the protein with all water molecules present, and similarly for the ligand–water term. The final (solvent–solvent) contribution would, however, be prohibitively expensive to evaluate in this manner.

The approximation we use is to assume that every two protein–solvent or ligand–solvent hydrogen bonds lost on complex formation correspond to one solvent–solvent hydrogen bond gained (the corresponding change in protein–ligand hydrogen bonds has already been assimilated in the $\mathbf{W}_{\mathbf{pl}}^{\mathbf{c}}$ term). One can calculate the change in the number of hydrogen bonds using HBPLUS.[6] One then multiplies the calculated number of new solvent–solvent hydrogen bonds by the typical hydrogen-bonded water–water interaction energy, calculated using BLEEP-2. We have written a computer program to generate all the water molecules within a possible interaction distance of the protein (i.e., within 8.0 Å of any protein atom), and then to remove those that make van der Waals clashes. The first shell of water molecules around the protein is, where possible, positioned using AQUARIUS2.[7,8]

One can also carry out similar calculations using BLEEP-1. In the case of the solvation-inclusive energies, however, there is a difficulty in that BLEEP-1 does not properly describe interactions involving water. Thus, one is unable to include solvation with BLEEP-1.

Use of BLEEP-2 on Test Systems

N—H . . . O=C HYDROGEN BOND

We test BLEEP-2 by evaluating the interaction energy of a system containing a typical N—H . . . O=C hydrogen bond by using three methods. First, we use BLEEP-2. Second, we utilize intermolecular perturbation theory (IMPT).[9] Third, we make use of the DMA+ model potential (which is a new name for a potential used previously by Moodie et al.[10] and based on distributed multipole analysis, DMA[11]). For the latter two approaches, the system used is formamide . . . formaldehyde, for which detailed 6-31G* quantum-chemical results are available.[12] For BLEEP-2, we wish to use protein-like atom types in our model. In formamide, the atom type[1,13] of the nitrogen is *0701*, whereas, in proteins and peptides, the nitrogen is type *0702*. Thus, we take *N*-methylformamide as our model to reproduce the desired atom types.

HUMAN BASIC FIBROBLAST GROWTH FACTOR–HEPARIN COMPLEXES

We carried out a number of calculations on a set of bFGF–heparin complexes. The calculations involved ranking a set of 3909 computer-generated decoy-binding orientations of a complex between bFGF and the hexasaccharide heparin, together with the crystal structure conformation taken from the PDB entry 1bfc.[14] The binding of heparin to fibroblast growth factors is important in the regulation of cell growth and differentiation and also in angiogenesis, wound healing, and cancer.[15] The 3909 decoy conformations were generated by the FTDOCK computer program,[16] with heparin treated as a rigid body. The distance between the atomic position in the test conformation and in the crystal structure is evaluated for each of the six anomeric C1 carbons in the hexasaccharide, and these six values are summed to produce a distance metric. This metric is used to measure the distance of each complex from the crystal structure conformation.

SET OF 90 PROTEIN–LIGAND COMPLEXES

We have, from a number of sources,[17–19] assembled a dataset of protein–ligand complexes in the PDB for which the experimental binding energies are known. This consists of 96 complexes, none of which were part of the dataset from which the potential was derived. A few of these turned out to be pathological cases for which sensible results could not be obtained. An example of this was the streptavidin–biotin complex, 1stp. This entry was found to be a major outlier by Morris et al.,[19] who felt that the difficulties were caused by the large conformational changes in the protein that accompany binding. Takamatsu et al.[20] experienced similar difficulties with a very closely related complex, which they ascribed to the presence of a plurality of possible binding modes. In all, we excluded six structures, leaving a set of 90 protein–ligand complexes, which are listed, together with those excluded, in Table I.

For these 90 complexes, we calculated interaction energies using each of the different protocols just described. At each level of energy calculation, we compared our calculated values with the experimental binding energies and evaluated the

TABLE I.
Set of 90 Protein–Ligand Complexes.

1abe	1abf	1add	1apv	1apw	1bll	1cbx	1cho	1cps	1dbb
1dbj	1dbk	1dbm	1dog	1etr	1ets	1ett	1fkb	1fkf	1gpy
1hef	1heg	1hew	1hri	1hvi	1hvj	1hvk	1hvl	1mfc	1nnb
1ola	1ppc	1pph	1rbp	1rgk	1rgl	1rpa	1tec	1tet	1thl
1tlp	1tmn	1ulb	2bop	2cpp	2dbl	2er6	2er7	2er9	2gbp
2gpb	2ifb	2kai	2ptc	2r04	2sec	2sni	2tgp	2xis	2ypi
3cpa	3er3	3gpb	3sgb	3tsl	4cpa	4gpb	4hmg	4hvp	4phv
4sga	4sgb	4tln	4tmn	4xia	5abp	5cpp	5gpb	5hvp	5sga
5tln	5xia	6tim	6tmn	7gpb	7hvp	8cpa	8gpb	9hvp	9icd

Six pathological cases were excluded from the original set of 96 complexes. These were: 1stp, 2er0, 4er4, 5er2, 6cpa, and 7cpa.

correlation coefficient. This we used as the principal measure of the success of BLEEP.

NINE SERINE PROTEINASE–INHIBITOR COMPLEXES

Zhang et al.[17] calculated binding energies for nine complexes between serine proteinases and polypeptide inhibitors (1cho, 1tec, 2kai, 2ptc, 2sec, 2sni, 2tgp, 3sgb, and 4sgb). As a further test of BLEEP-2, we calculated (pseudo-gas-phase) energies for each of these complexes and compared the correlations with experiments achieved with those of Zhang et al.[17] Ranking the interaction energies of a series of similar complexes is an important task for BLEEP to perform well, given that it was designed to be applicable to the drug discovery process.

Results

N—H . . . O=C HYDROGEN BOND

The geometry of the complex used is shown in Figure 1: the angle α is set to 42°. The variation of energy with distance, Figure 2, shows that BLEEP-2 gives a minimum at a N . . . O distance similar to quantum-chemical methods (around 2.9 Å). The well is less deep than those calculated by the IMPT and DMA+, but we would not expect the energies calculated by a PMF to be quantitatively comparable with quantum-chemical values. We note that BLEEP-2 becomes repulsive at intermediate range (3.3–6.0 Å). This is a consequence of the incorporation of entropy in what is effectively a Helmholtz free energy, ΔA,[4,5] and was also seen by Sippl in his PMF study of hydrogen bonding.[4] Sippl describes this as a barrier between almost isoenergetic short-range (hydrogen bonded) and long-range (nonbonded) states.[4] We obtained a deeper well than he did, probably because we considered the change in distances between all pairs of atoms (including polar hydrogens) in the fragments concerned, rather than just the nitrogen and oxygen, although we also note that our study was done for protein–ligand and his for protein–protein interactions. Given that the success of Jones et al.[21] in predicting ligand binding was based largely on correct prediction of hydrogen bonding and other polar interactions, we are pleased to have seen hydrogen bonds corresponding to significant wells in BLEEP. We also found that the lack of atom–atom anisotropy in our functions did not introduce any unrealistic angular behavior.

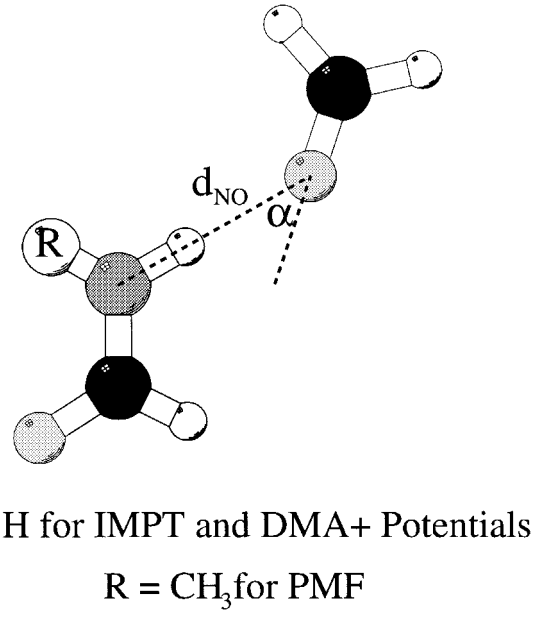


FIGURE 1. The molecules and geometric variables used in our calculations of N—H . . . O=C hydrogen bond energies. The variables are the N . . . O distance (d_{NO}) and the in-plane angle (α). The sign of α is positive in the conformation illustrated; the calculations illustrated in Figure 2 were carried out with α equal to +42°. For IMPT and DMA+ calculations, formamide was used (R = H). For BLEEP-2, we model protein-like atom types. In formamide, the atom type[1,13] of the nitrogen is *0701*, whereas, in proteins and peptides, it is *0702*. Thus, we use *N*-methylformamide (R = CH_3) as our model in this case to reproduce the desired nitrogen atom type, *0702*.

HUMAN BASIC FIBROBLAST GROWTH FACTOR–HEPARIN COMPLEXES

Figure 3 shows the pseudo-gas-phase energies, calculated using BLEEP-2, plotted against the metric measuring the distance between the test conformation and that observed in the heparin hexasaccharide crystal structure (Fig. 4). The BLEEP-2 energy function successfully identified the crystal structure conformation,[14] marked by a square in Figure 3, as having a lower energy than any of the 3909 decoy conformations. This also corresponds to ''site 1,'' identified by Ornitz et al.,[15] who found multiple bFGF binding sites for nonsulfated, heparin-derived, di- and trisaccharides. There was a large energy gap between this conformation and any of the decoys and only two decoy conformations were

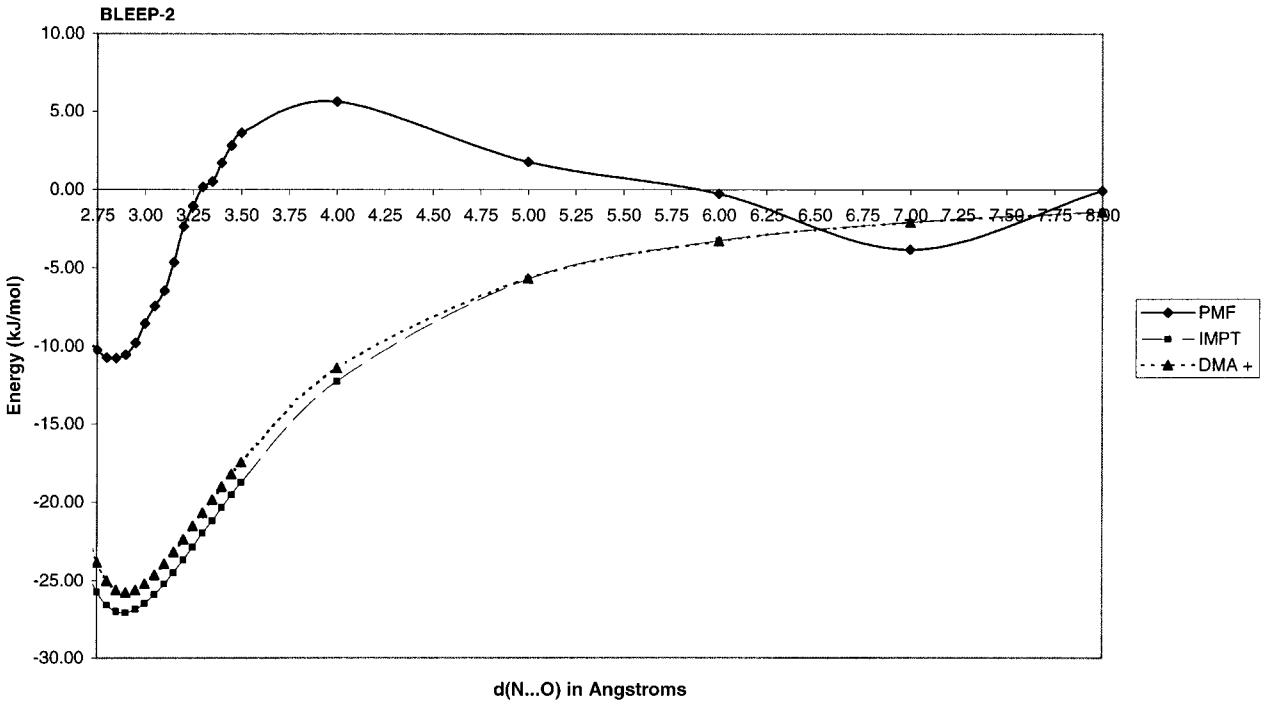


FIGURE 2. Hydrogen bond energy variation with N . . . O distance for BLEEP-2, compared with IMPT calculations[9,12] and the DMA+ model potential[10] based on distributed multipole analysis.[11] For the latter two approaches, the hydrogen bond taken is from formamide . . . formaldehyde.[12] For BLEEP-2, however, *N*-methylformamide replaces formamide, so that the atom types are protein-like. The in-plane angle (α) is set to 42°.

found within 135 kJ/mol. The nearest-to-native of the decoy conformations, marked by a circle in Figure 3, had the second lowest energy among the 3909 decoys. The lowest energy decoy structure is indicated by a triangle in Figure 3. For this conformation, the heparin hexsaccharide overlaps the site denoted 2′ by Ornitz et al. Several of the interacting residues observed in this decoy conformation (Lys78, Arg73, Arg82) correspond directly to those (77, 72, 81) identified in disaccharide binding at site 2′. The crystal structure and two lowest energy decoys had significant stability gaps between their energies and the energy continuum of the main body of decoy structures.[22,23] Most of the decoy complex energies fell within a fairly limited energy range. As many as 3008 out of 3909 values (77.0%) were between +200 and −200 kJ/mol and 3455 (88.4%) between +300 and −300 kJ/mol. Figure 3 shows only the 1616 decoys with negative energies. In comparison with BLEEP, CHARMM electrostatic calculations were notably less successful in picking out close-to-native structures (M. Forster, unpublished results).

SET OF 90 PROTEIN–LIGAND COMPLEXES

The correlation coefficients obtained for the set of 90 protein–ligand complexes are shown in Table II. The pseudo-gas-phase interaction energies calculated with BLEEP-2 give a correlation coefficient with experiment of 0.74, as illustrated in Figure 5a. If we exclude the imported reference potential (described in the accompanying article and taken from Ng et al.[24]) from all calculated energies, the correlation coefficient is still 0.74. This suggests that there were few van der Waals clashes in the structures.

We have also obtained results with our solvation-inclusive algorithm. The correlation coefficient achieved was 0.63 (Fig. 5b). Thus, the protocol for incorporating solvation did not improve the accuracy of the results. Given the approximations involved, especially in the water–water term, this is not a great surprise.

When we used BLEEP-1 to calculate the solvent-exclusive interaction energies, we obtained a correlation coefficient of 0.67. Thus, BLEEP-1 fared less

well than BLEEP-2 in this test, as indeed it had done in various preliminary trials.[1] We note that the absolute magnitudes of the ''energies'' predicted by our method were considerably larger than the experimental values.

NINE SERINE PROTEINASE–INHIBITOR COMPLEXES

BLEEP-2 gave a correlation coefficient of 0.71 with the experimental interaction energies quoted by Zhang et al.[17] This compares with the very similar value of 0.70 achieved by Zhang et al.'s own theoretical method, which was based on atomic desolvation energies.[17] The inclusion of solvation, however, reduced the correlation coefficient to 0.54.

Discussion

The trials we have carried out have tested quite different aspects of BLEEP-2's capabilities. In looking at the N—H...O=C hydrogen bond, we have been considering a short-range interaction involving only a handful of atoms. BLEEP-2 produced a minimum at almost exactly the desired distance, demonstrating that it favored such hydrogen bonds, and also behaved reasonably at medium and long range.

The ranking of different bFGF–heparin binding conformations tested another important feature: the capacity to differentiate between different binding geometries for the same complex. The particular success of BLEEP-2 in this context encour-

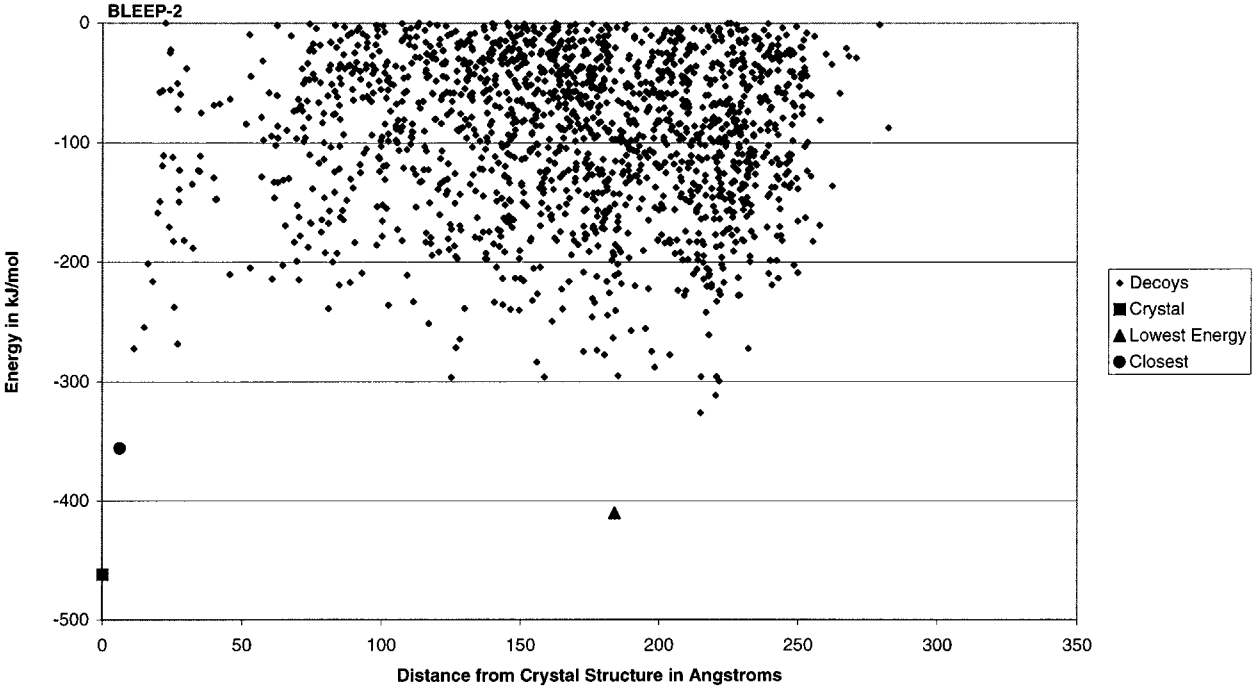


FIGURE 3. The calculated PMF energy plotted against a distance metric (see text) for the 1616 (out of a total of 3909) decoy bFGF–heparin complex geometries that have negative energies and for the experimental crystal structure.[14] BLEEP-2 successfully assigns a lower energy to the crystal structure, marked by a square and designated as site 1 by Ornitz et al.,[15] than to any of the 3909 alternative geometries. The computer-generated structure closest to the experimental conformation is given the second lowest energy of the 3909 decoys, and is marked by a circle. The lowest in energy of these 3909 structures is marked by a triangle; this corresponds to another experimentally known binding site, labeled 2′ by Ornitz et al.[15] The decoy conformations were generated using FTDOCK,[16] which utilizes the Fourier correlation method of Katchalski–Katzir[25] effectively to perform a systematic and efficient search of the three translational and three rotational degrees of freedom. We used an angular step size of 10° and a surface thickness of 1.2 Å, with the three best orientations being saved from each translational scan. Heparin was treated as a rigid body and only a coarse scan was carried out.

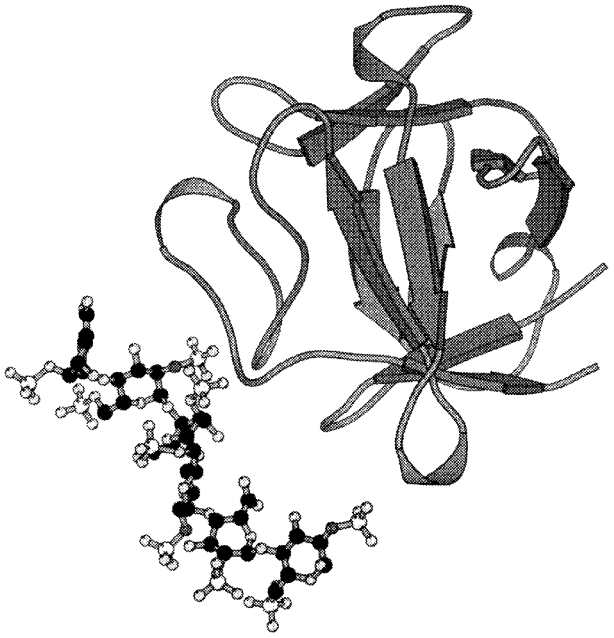


FIGURE 4. MOLSCRIPT[26] diagram of heparin complexed with bFGF. The conformation shown here is from the crystal structure, PDB code 1bfc.[14] It is picked out with a square in Figure 3.

ages us that it is likely to be able to pick out binding conformations from decoys. We can therefore expect that, given a protein target and a drug molecule ligand, BLEEP-2 will be likely to successfully find the correct binding mode, or at least to rank the correct mode highly among the possibilities.

The 90 protein–ligand complexes provided another kind of test. Here, we were comparing the relative interaction energies of a set of quite different complexes. This is clearly a more difficult task than ranking different conformations of the same complex, and the performance of BLEEP-2 proved to be encouraging. Excluding the imported reference potential had only a small effect on the correlation for these high-quality crystallographic structures. We would expect the absence of repulsive wall functions to be more serious for structures with van der Waals clashes, such as inadequately refined structures or poorly docked models.

It is, however, disappointing that the solvent-inclusive protocol actually showed a poorer correlation with experiment than the pseudo-gas-phase one. This suggests that the approximations inherent in our model of solvation allow an accumulation of errors that outweighs the accuracy gained from considering solvation effects. We are aware that the water–water interaction energy term, based on counting hydrogen bonds, may be problematic.

The nine serine proteinase–inhibitor complexes tested the ability of BLEEP-2 to discriminate between members of a series of similar complexes, an important attribute in the context of the drug design process. It is encouraging that BLEEP-2 matched the performance of Zhang et al.'s method[17] in achieving a respectable correlation with experiment.

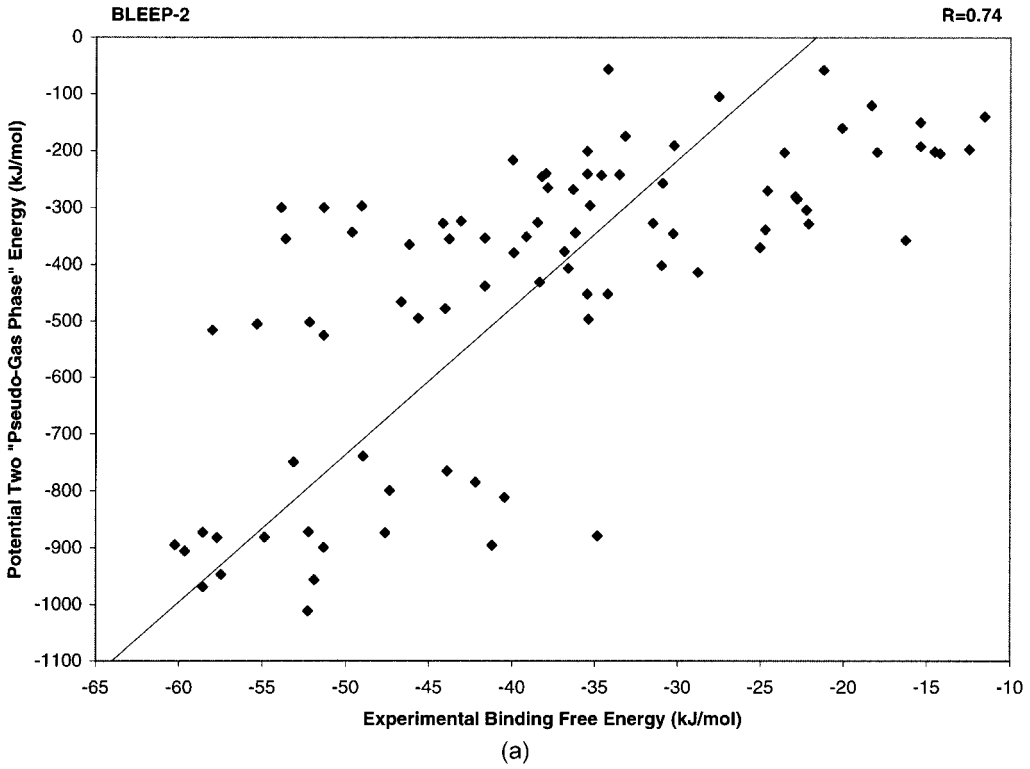
TABLE II.
Correlation Coefficients of Calculated and Experimental Binding Energies for 90 Protein–Ligand Complexes.

Protocol	BLEEP-2	BLEEP-1
Pseudo-gas phase	0.74	0.67
Excluding reference function	0.74	0.65
Solvation	0.63	n/a

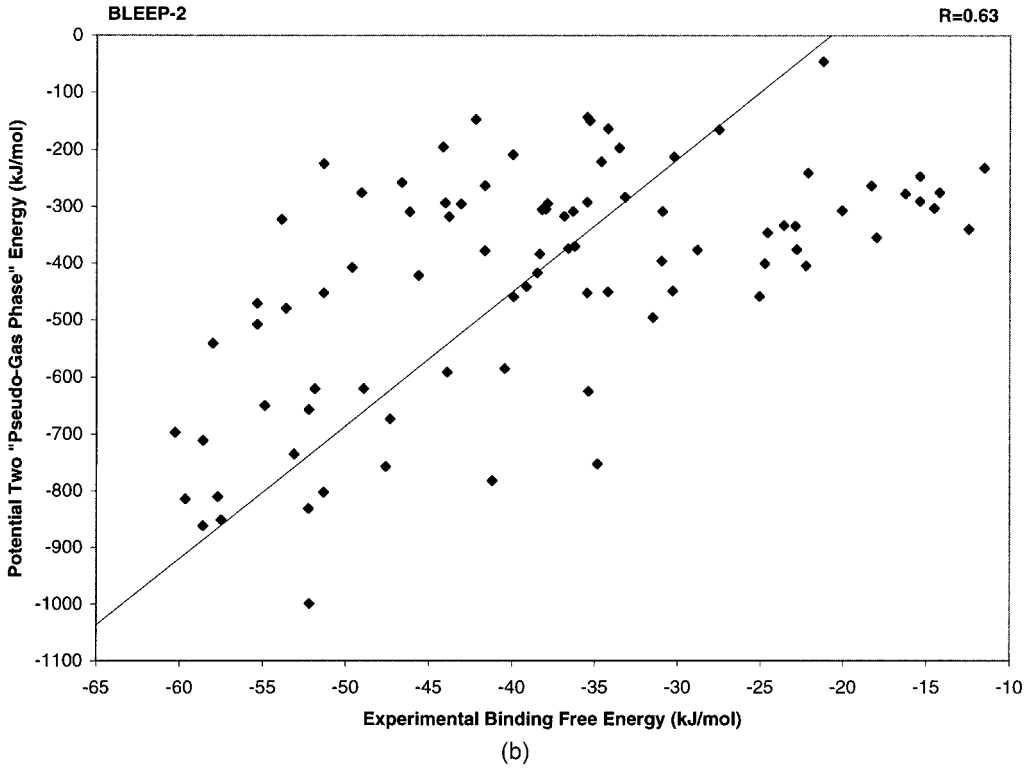
Conclusions

Overall, BLEEP-2 performed encouragingly in the range of tests presented here. The results on the small hydrogen-bonded complex suggest that BLEEP-2 gives an adequate description of hydrogen bonding. In the study of protein–heparin complex energies, BLEEP-2 assigns the true crystal structure a lower energy than any of 3909 alternatives, and the closest to native of these is ranked second of the decoys. The first ranked decoy corresponds to another known experimental binding geometry. Thus, BLEEP-2 performs excellently in identifying low-energy binding modes from decoy conformations for this protein–ligand complex. The correlation coefficient of 0.74 obtained for the calculated and experimental energies of 90 protein–ligand structures is an important result. It shows that BLEEP-2 can perform well in the difficult area of ranking the interaction energies of diverse complexes. The study of serine proteinase–inhibitor complexes demonstrates the PMF's capacity to discriminate between similar complexes.

BLEEP was designed with drug design in mind. We hope to be able to use it to rank the relative energies of a series of candidate drug molecules interacting with a given target. We believe that the



(a)



(b)

FIGURE 5. (a) Correlation between the experimental binding energies and pseudo-gas-phase values calculated using BLEEP-2. The dataset comprises 90 structures from the PDB.[2] The best-fit line is generated by means of principal component analysis of the data. The correlation coefficient is 0.74. (b) Correlation between the experimental binding energies and solvation-inclusive values calculated using BLEEP-2: the correlation coefficient here is 0.63.

results obtained here demonstrate that this is likely to be successful. Indeed, the bFGF–heparin results suggest that the PMF may be able to identify the correct binding mode in the absence of a protein–ligand complex structure.

Acknowledgments

This is a publication from the BBSRC Structural Biology Centre in Birkbeck College and University College London.

References

1. Mitchell, J. B. O.; Laskowski, R. A.; Alex, A.; Thornton, J. M. J Comput Chem (this issue).
2. Bernstein, F. C.; Koetzle, T. F.; Williams, G. J. B.; Meyer, E. F.; Brice, M. D.; Rodgers, J. R.; Kennard, O.; Shimanouchi, T.; Tasumi, M. J Mol Biol 1977, 112, 535.
3. Mitchell, J. B. O. Price, S. L. Chem Phys Lett 1991, 180, 517.
4. Sippl, M. J. J Mol Biol 1996, 260, 644.
5. Sippl, M. J. Ortner, M.; Jaritz, M.; Lackner, P.; Flöckner, H. Fold Des 1996, 1, 289.
6. McDonald, I. K.; Thornton, J. M. J Mol Biol 1994, 238, 777.
7. Pitt, W. R.; Murray-Rust, J.; Goodfellow, J. M. J Comput Chem 1993, 14, 1007.
8. Goodfellow, J. M.; Pitt, W. R.; Smart, O. S.; Williams, M. A. Comput Phys Commun 1995, 91, 321.
9. Hayes, I. C.; Stone, A. J. Molec Phys 1984, 53, 83.
10. Moodie, S. L.; Mitchell, J. B. O.; Thornton, J. M. J Mol Biol 1996, 263, 486.
11. Stone, A. J. Chem Phys Lett 1981, 83, 233.
12. Mitchell, J. B. O.; Price, S. L. J Comput Chem 1990, 11, 1217.
13. Mitchell, J. B. O.; Alex, A.; Snarey, M. J Chem Inf Comput Sci (accepted for publication).
14. Faham, S.; Hileman, R. E.; Fromm, J. R.; Lindhardt, R. J.; Rees, D. C. Science 1996, 271, 1116.
15. Ornitz, D. M.; Herr, A. B.; Nilsson, M.; Westman, J.; Svahn, C.-M.; Waksman, G. Science 1995, 268, 432.
16. Gabb, H. A.; Jackson, R. M.; Sternberg, M. J. E. J Mol Biol 1997, 272, 106.
17. Zhang, C.; Vasmatzis, G.; Cornette, J. L.; DeLisi, C. J Mol Biol 1997, 267, 707.
18. Böhm, H.-J. J Comput-Aid Mol Design 1994, 8, 243.
19. Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Huey, R.; Hart, W. E.; Belew, R. K.; Olson, A. J. J Comput Chem 1998, 19, 1639.
20. Takamatsu, Y.; Itai, A. Proteins 1998, 33, 62.
21. Jones, G.; Willet, P.; Glen, R. C.; Leach, R.; Taylor, R. J Mol Biol 1997, 267, 727.
22. Verkhivker, G. M.; Rejto, P. A. Proc Natl Acad Sci USA 1996, 93, 60.
23. Rejto, P. A.; Verkhivker, G. M. Proteins 1997, 28, 313.
24. Ng, K.-C.; Meath, W. J.; Allnatt, A. R. Molec Phys 1979, 37, 237.
25. Katchalski-Katzir, E.; Shariv, I.; Eisenstein, M.; Friesem, A. A.; Aflalo, C.; Vakser, I. A. Proc Natl Acad Sci USA 1992, 89, 2195.
26. Kraulis, P. J. J Appl Cryst 1991, 24, 946.